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THE DILATABLE RING CANALS OF THE OVARIAN CYSTOCYTES OF HABROBRACON JUGLANDIS

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The insects possessing polytrophic, meroistic ovarioles belong to the adephagous Coleoptera, the Dermaptera, Siphunculata, Neuroptera, Mecoptera, Lepidoptera, Diptera, and the Hymenoptera (Imms, 1957). In such insects the oocyte is a member of a cluster of sister cells. The other cells of the cluster function as nurse cells. Interconnections have been observed between the nurse cells and the oocyte in insects belonging to the Hymenoptera, Coleoptera, Lepidoptera, and Diptera (Brown and King, 1964; King and Aggarwal, 1965). In the fruit fly, *Drosophila melanogaster*, each of the egg chambers that reside in the vitellarium contains an oocyte and fifteen nurse cells. The oocyte and its interconnected nurse cells are fourth generation descendants of a single cell, a cystoblast, that resides in the germarium. The interconnected cells formed by the division of a cystoblast are called cystocytes (Brown and King, 1964). Sister cystocytes are connected by canals, each of which is surrounded by a ring-shaped rim. A stream of nurse cell cytoplasm rich in mitochondria, lipid droplets, and ribosomes passes through these ring canals and into the ooplasm (King, 1960; Cummings and King, 1969). It has been shown that the vast majority of ribosomes found in the mature *Drosophila* oocyte are derived from sister nurse cells (Koch, Smith and King, 1967; Dapples and King, 1970).

This paper presents the results of an ultrastructural study of the ring canals found in the germarial region of an ovariole from the parasitic braconid wasp, *Habrobracon juglandis* (Ashmead). This species is also referred to in the literature as *Microbracon hebetor* and *Bracon hebetor*. By studying serial transverse sections through several canals, it was possible to arrive at an understanding of the three-dimensional ultrastructure of the canal rim and to demonstrate that the rim is constructed in a manner that allows it to dilate.

Materials and Methods

Wild type female wasps from Whiting stock 33 reared at 30° C upon larvae of the Mediterranean flour moth, *Ephestia* (= *Angasta*) *kühniella*, provided the ovarian material. Ovarioles from newly emerged adults were fixed in a 0.2 *M* sodium cacodylate buffer solution (pH 7.45) containing 4% glutaraldehyde. The tissue was passed through six, thirty-minute changes of the glutaraldehyde fixative at 4° C. Next the tissue was washed by passing through five-, one-hour changes of glutaraldehyde-free buffer. After washing, the tissue was postfixed for two hours in cold 1% OsO_4 in the same buffer. Rapid dehydration in an ethanol series followed, and after a transfer to propylene oxide, the ovarioles were infiltrated with

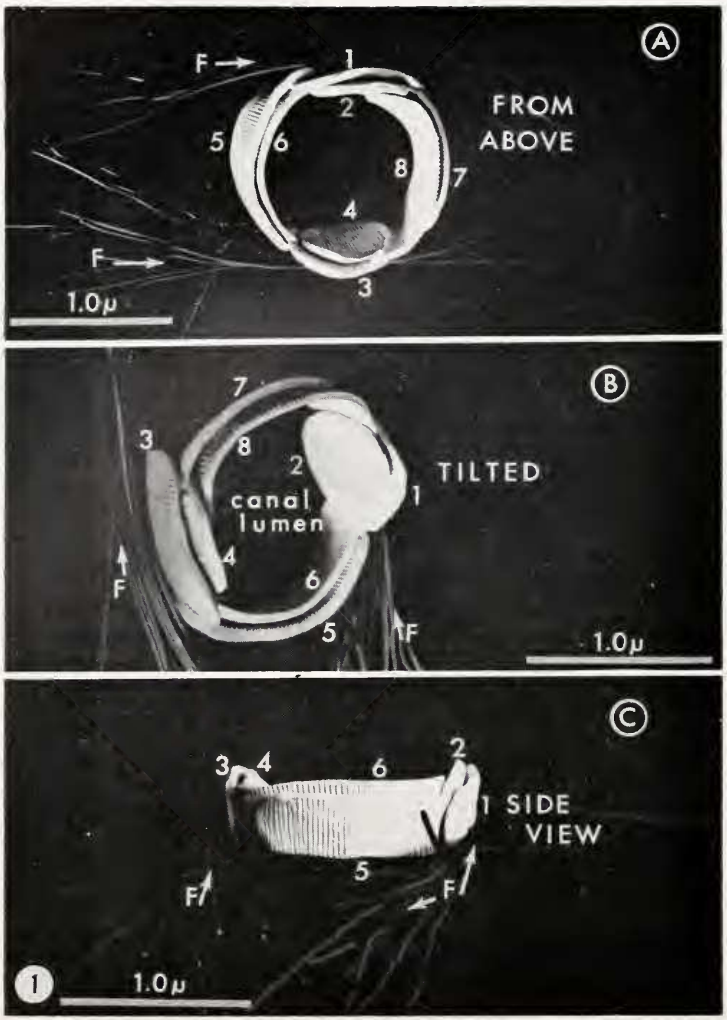

FIGURE 1. Photographs of the Polyform model of the system of semiannular leaves which surround a ring canal of *Habrobracon jnglandis*. The model has been photographed after being tilted in various planes. The ring is made up of paired sets of short, broad leaves (1, 2 and 3, 4) and long, narrow leaves (5, 6 and 7, 8). Each of the eight leaves is made up of a parallel array of short microtubules. These are represented by parallel grooves carved into the outer surface of the Polyform leaves. Filaments (F), represented by bundles of insulated wires, extend into the cytoplasm in both directions from the short broad leaves. The filaments extending from the long narrow leaves are not included in the model.

Maraglas and polymerized according to the method of Erlandson (1964). One block was selected for sectioning because of the advantageous orientation of the germarium. Approximately 200 serial, longitudinal sections, each about 70 mμ thick, were cut using an LKB Ultrotome III equipped with a glass knife. Sections were picked up in groups of 8–10 upon Formvar-carbon coated, one-hole copper

grids using the LKB section collector-stereoscope assembly. The sections were stained by successive immersions in saturated, aqueous solutions of uranyl acetate (10 min) and lead citrate (5 min) according to the procedure of Frasca and Parks (1965).

Approximately 970 electron micrographs were taken with a Hitachi HU 11A microscope operated at 50 KV. Composite electron micrographs were made from the overlapping prints representing areas from each section at a magnification of

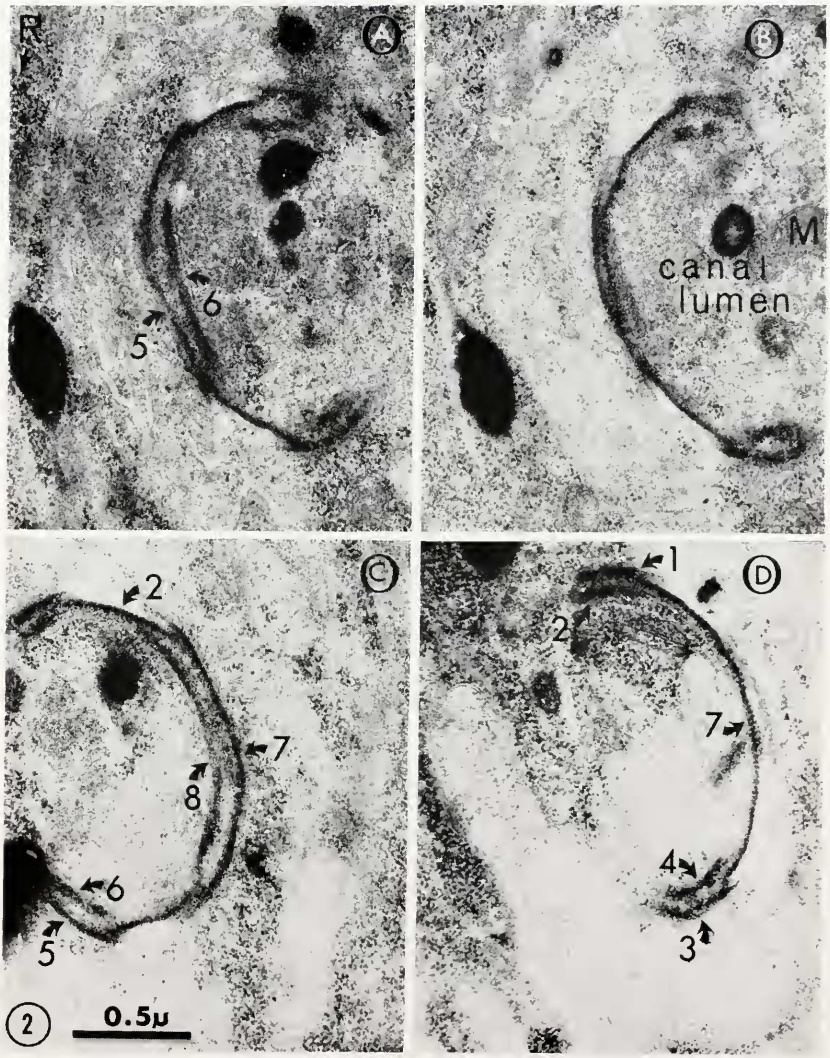

FIGURE 2. Electron micrographs of four serial thin sections from a ring canal found in a cluster of cystocytes located in the posterior region of the germarium. The leaves are labeled as in Figure 1. The cytoplasm contains ribosomes (R) and mitochondria (M).

11,000 ×. The interconnections between cystocytes were localized in 156 such composites. Transversely-sectioned ring canals were observed on rare occasions, and three such canals from cystocyte clusters located within the anterior, middle and posterior portions of the germarium were chosen for study at higher magnification. The appropriate negatives were selected, and enlargements were made using Kodak positive sheet film. The result was a series of positive transparencies showing the canals at 38,000 ×. The transparencies from serial sections were oriented one above the other and viewed simultaneously in order to grasp the three-dimensional morphology of the canal rim. Eventually a 50,000 × model was made of a canal rim using the malleable plastic, Polyform (see Koch and King, 1969 for the details of Polyform model construction).

Results and Conclusions

Each canal connecting sister cystocytes in the *Habrobracon germarium* is in the shape of a laterally flattened right cylinder with an altitude ranging between 0.4 and 0.7 μ (Fig. 1). When viewed in a perfect transverse section the canal from which the model was constructed formed an ellipse with major and minor axes of 1.3 and 1.0 μ, respectively (Fig. 2). The rim surrounding each canal was made up of eight leaves arranged in four pairs. The leaves of each pair overlapped with the leaves of adjacent pairs. The arrangement of the leaves is easily grasped after viewing Figure 1. When the ring is sectioned in the longitudinal or frontal plane one obtains the image seen in Figure 3.

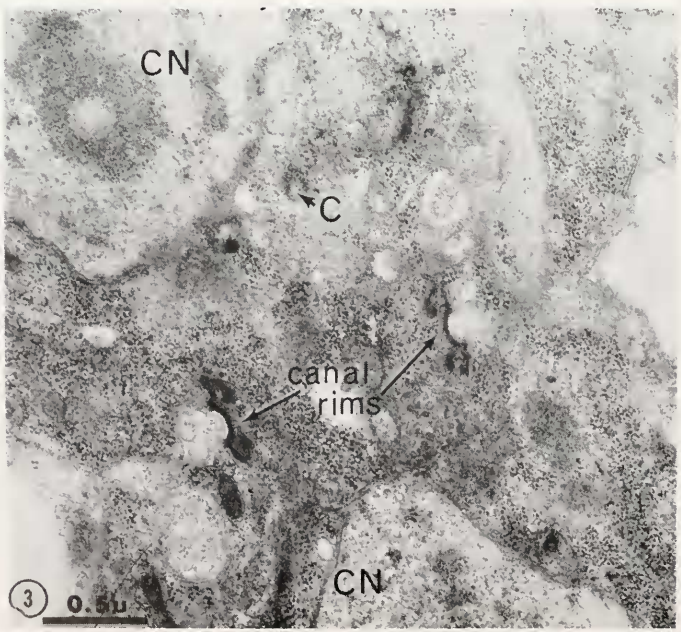

Figure 3. A canal rim cut in longitudinal (or frontal) section. The vast majority of the canal rims seen in electron micrographs are sectioned in this manner: C, centriole; CN, nuclei of sister cystocytes.

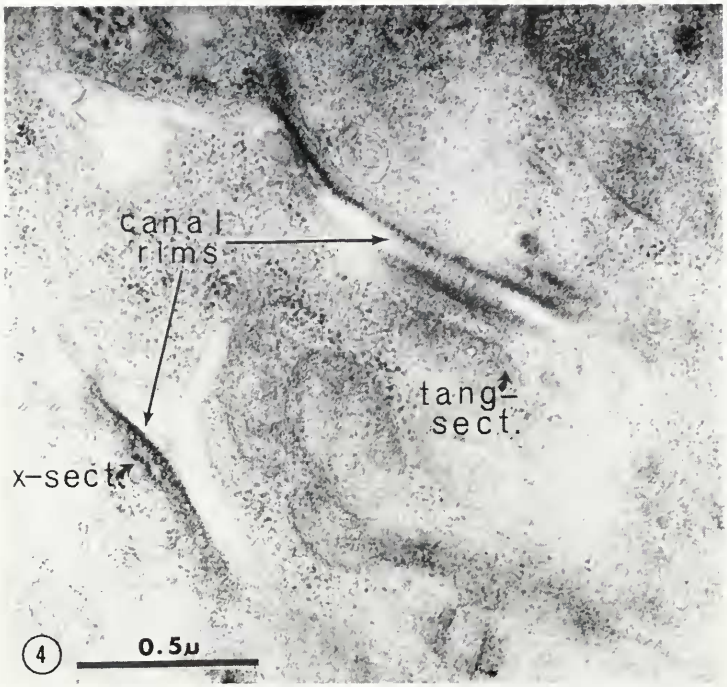

FIGURE 4. Two canal rims; one cross-sectioned, the other cut tangenitally. The parallel orientation of the microtubules in a leaf can be seen in the upper rim.

The short broad leaves have a length of $\sim 1.1\ \mu$ and a width of $\sim 0.7\ \mu$. The long narrow leaves have a length of $\sim 2.0\ \mu$ and width of $\sim 0.4\ \mu$. The short and long leaves are ~ 0.05 and $0.04\ \mu$ thick, respectively. Each leaf is made up of a monolayer of short, parallel microtubules. These are clearly seen in Figures 4 and 5. The mean outer diameter of each microtubule is $\sim 20\ m\mu$, and each is separated from its neighbor by a space $\sim 10\ m\mu$ wide. From the above dimensions we calculate each short and long leaf contain about 40 and 70 microtubules, respectively. These are embedded in an electron-dense matrix.

In Figures 6 and 7 drawings are presented of two cross-sectioned canals. Both are magnified to the same degree, but one has a greater circumference. The dilation has been accompanied by a change in the orientation of the long, narrow leaves. Obviously leaves 5 and 7 have slid apart from leaves 6 and 8. The pairing pattern of the short, broad leaves seems unchanged.

Filamentous attachments are also observed in the cytoplasm, surrounding the leaves of the rim. These filaments are much longer than those embedded in the leaves, but their diameters are about the same. The orientations of these membranes are shown in Figure 8. They seem to attach to all the leaves, but the short, broad leaves seem to possess denser clusters of these cytoplasmic filaments. An electron micrograph illustrating these filamentous attachments lying perpendicular to the rows of microtubules in a canal rim, is presented in Figure 9.

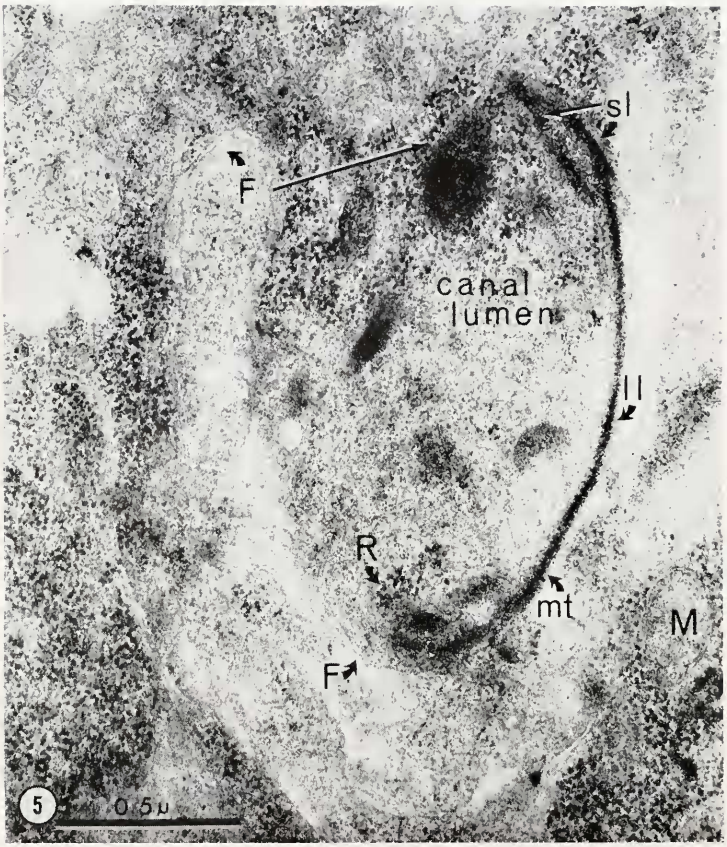

FIGURE 5. Detail of the array of microtubules (mt) in a long leaf (ll) intersecting two shorter leaves (sl) cut in cross-section; F, cytoplasmic filamentous attachments to the canal leaves; R, ribosomes; M, mitochondrion.

DISCUSSION

Koch and King (1969) have shown in *Drosophila melanogaster* that the ring canal rim is completed *prior* to the appearance of the plasma membrane to which the canal rim is later attached. Koch and King suggest that the canal rim is somehow derived from a "mid-body." This is a dense, disc-shaped structure which is commonly observed in the middle of the ephemeral cytoplasmic bridge that connects sister cells in late telophase. In other species ultrastructural studies have shown that the mid-body consists of a disc of electron-dense material in which a bundle of microtubules is embedded (Buck and Tisdale, 1962; Allenspach and Roth, 1967; Rosai *et al.*, 1969). The finding of microtubules embedded in the leaves surrounding the ring canals of *Habrobracon* strengthens the hypothesis that the rim elements are derived from a mid-body.

Koch and King's electron micrographs demonstrate that in *Drosophila* after the ring is formed there is produced along the plane of division a plaque of intercon-

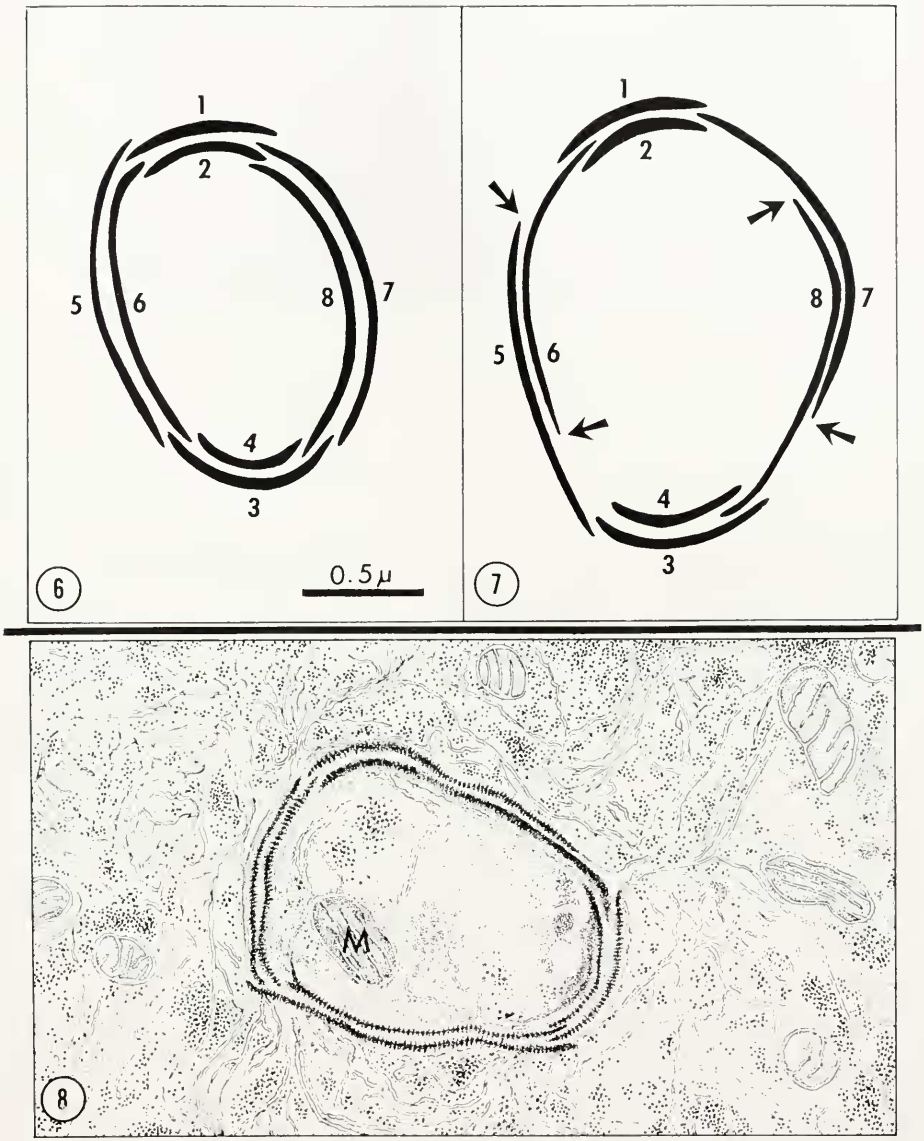

FIGURE 6. An outline drawing of a cross-sectioned canal showing the orientation of the eight leaves which form the rim.

FIGURE 7. An outline drawing of a cross-sectioned canal which is dilated relative to the canal shown in Figure 6.

FIGURE 8. A drawing illustrating the orientation of the cytoplasmic filamentous attachments which surround the canal relative to the microtubules comprising the leaves; M, mitochodrion.

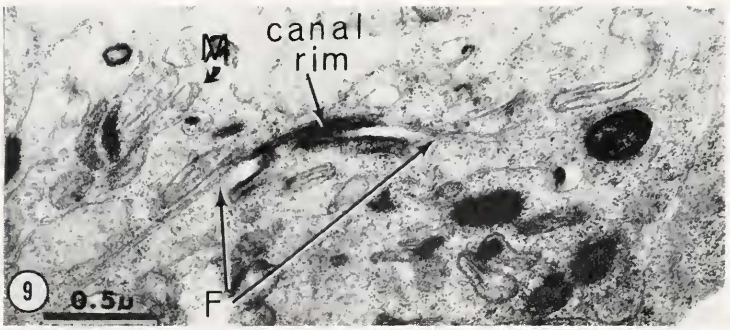

FIGURE 9. An electron micrograph of a glancing section through the upper portion of the canal rim containing short leaves. Cytoplasmic filaments (F) extend in both directions from the surface of the leaves; M, mitochondrion.

nected vesicles and tubules and that these later coalesce to form continuous sheets of membrane which segregate the cytoplasms of the sister cells, except in the region enclosed by the ring. As development proceeds the cystocytes increase in volume, and each canal dilates, its rim becomes thicker, and the inner circumference of the rim becomes coated with a thick deposit having different cytochemical properties than the rim itself. These findings support the hypothesis that in *Drosophila* the canal rim is a metabolically active organelle capable of undergoing morphological changes of functional significance.

In *Habrobracon* it is also obvious that each canal rim dilates as the cystocytes develop. The mechanism of dilation seems to involve the sliding apart of specific paired leaves. A decision as to whether or not the attached cytoplasmic filaments play any role in this movement must await further investigations.

Canals similar to those connecting insect cystocytes have been found between young oocytes in the brine shrimp, *Artemia salina* (Anteunis, Fautrez-Firelefyn, and Fautrez, 1966), between the oocyte and its accessory cells in the polychaete, *Diopatra cuprea* (Anderson and Huebner, 1968), and between oocytes in the ovary of the rat (Franchi and Mandl, 1962), the hamster (Weakley, 1967), the rabbit (Zamboni and Gondos, 1968), and the mouse (Ruby, Dyer and Skalko, 1969). In the case of rodents and many other higher mammals a huge majority of the early oocytes ultimately degenerate. Davidson (1968) has suggested that, as in the meroistic system of insects, most of the interconnected oocytes actually function as nurse cells and degenerate once this task is accomplished. Thus throughout the animal kingdom during oogenesis sister germ cells are commonly found to be connected by cytoplasmic canals at an early stage in their development. Time will tell as to whether dilatable canal rims of the type described here are restricted to a few insect species or are widespread throughout the animal kingdom.

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Summary

The canals connecting the ovarian cystocytes of *Habrobracon juglandis* are enclosed by a ring made up of eight leaves. Each leaf is composed of a monolayer of between 40 and 70 short parallel microtubules which may be derived from the mid-body. The sliding of certain leaves past one another allows the ring to dilate as the cystocyte grows.

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